Published in "Food Chemistry 133(4): 1435–1440, 2012"
which should be cited to refer to this work.

Oxidation of sterols: Energetics of C–H and O–H bond cleavage

Jozef Lengyel [a,b], Ján Rimarčík [c,*], Adam Vagánek [c], Juraj Fedor [d], Vladimír Lukeš [c], Erik Klein [c]

[a] J. Heyrovský Institute of Physical Chemistry, v.v.i., Academy of Sciences of the Czech Republic, Dolejškova 3, CZ-182 23 Prague 8, Czech Republic
[b] Department of Physical Chemistry, Institute of Chemical Technology, Technická 5, CZ-166 28 Prague 6, Czech Republic
[c] Institute of Physical Chemistry and Chemical Physics, Slovak University of Technology, Radlinského 9, SK-812 37 Bratislava, Slovakia
[d] Department of Chemistry, University of Fribourg, Chemin du Musée 9, CH-1700 Fribourg, Switzerland

Phytosterols, as components of human diet, received much attention because of their cholesterol-lowering and antioxidant properties. We have theoretically studied sterols oxidation in terms of O–H and C–H bond dissociation enthalpies (BDE). In 17 Δ^5- and Δ^7-sterols, BDEs were obtained for reported sites of oxidation attack. Obtained results indicate that Δ^7-sterols are more susceptible to oxidation attack in comparison to Δ^5-sterols. In sterol nuclei, the lowest BDE was found for C7–H bond in Δ^5-sterols and for C14–H in Δ^7-sterols. When Δ^5-sterol has a C=C double bond in the side chain, the lowest BDEs are usually found for C–H bonds in α-positions to this bond. The homolytic cleavage of hydroxyl O–H bond requires larger energy in comparison to the studied C–H bonds. We have shown that the C–H bonds with lowest BDE values actually correspond to the dominant sites of oxidation attack.

1. Introduction

Phytosterols (plant sterols) are triterpenes representing important structural components of plant membranes. Free phytosterols stabilize phospholipid bilayers in plant cell membranes. Their structure and function is analogous to cholesterol in animal cell membranes. Most phytosterols contain 28 or 29 carbon atoms and one or two C=C bonds, typically one in sterol nucleus and the second one may be present in the alkyl side chain (see Figs. 1 and 2). Phytosterols having a double bond between C5 and C6 carbons in sterol nucleus are called Δ^5 phytosterols. Another group of phytosterols present in plants of certain families (Moreau, Whitaker, & Hicks, 2002) has a double bond between C7 and C8 carbons. These are referred to as Δ^7 phytosterols. More than 200 different types of phytosterols have been reported in plants.

The commonly consumed phytosterols are Δ^5-sitosterol, Δ^5-stigmasterol, and Δ^5-campesterol, which are predominantly supplied by vegetable oils (Piironen, Lindsay, Miettinen, Toivo, & Lampi, 2000). In last 10–15 years, phytosterols, as components of human diet, received much attention because of their cholesterol-lowering properties (García-Llatas & Rodríguez-Estrada, 2011; Moreau et al., 2002). They may protect against heart disease, therefore they have been recently incorporated into a growing spectrum of functional foods. Sterols are added to margarines, edible oils, dairy products, bakery products, sausages, fruit juices and snack bars (García-Llatas & Rodríguez-Estrada, 2011; Moreau et al., 2002).

Oxidation decreases consumer acceptability of foods by producing low molecular weight off-flavour compounds, as well as by lowering the nutritional value of foods (Choe & Min, 2009). Antioxidants naturally occurring or added to foods are able to significantly inhibit (slow down) the process of oxidation. It has been found that phytosterols present in edible oils show an antioxidant effect at elevated or frying temperatures (Gordon & Magos, 1983; Moreau et al., 2002; Sims, Fioriti, & Kanuk, 1972; Wang, Hicks, & Moreau, 2002). Moreover, several works (Vivacons & Moreno, 2005; Yoshida & Niki, 2003) reported an antioxidant effect of phytosterols against lipid peroxidation. On the other hand, many in vitro studies have shown that cholesterol oxides have cytotoxic, mutagenic and atherogenic activities, induce apoptosis and are potent regulators of cholesterol metabolism. Thus, they may have harmful effects on human health (Schroepfer, 2000). Due to structural similarities between phytosterols and cholesterol, plant sterols are also susceptible to oxidation. This, however, may be a serious problem in foods rich in sterols, and especially in sterol-enriched foods.

In general, oxidation of sterols is a free radical chain reaction that starts with the formation of hydroperoxides. The slowest step of the process is the abstraction of hydrogen atom (hydrogen atom transfer) from the oxidation attack site in a sterol molecule. The oxidation proceeds through several reaction pathways, where hydroxy, keto and epoxy compounds represent the main oxidation

* Corresponding author.
E-mail address: jan.rimarcik@stuba.sk (J. Rimarčík).

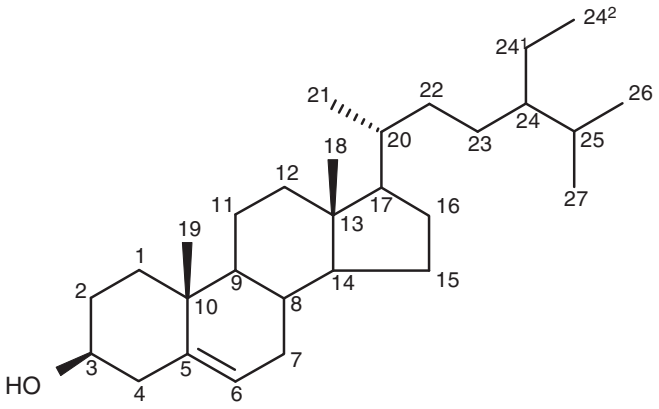

Fig. 1. Atom numbering in sterols according to IUPAC.

products. For cholesterol, more than 80 oxidation products have been identified (Lampi, Juntunen, Toivo, & Piironen, 2002; Schroepfer, 2000; Smith, 1987, 1996). The major products of Δ^5-stigmasterol, Δ^5-campesterol and Δ^5-sitosterol thermooxidation (180 °C, 24 h) are 7-hydroxy, 5,6-epoxy and 7-keto compounds. Oxidation at the side chain C25 carbon also took place (Lampi et al., 2002). Oehrl, Hansen, Rohrer, Fenner, and Boyd (2001) studied the oxidative stability of Δ^5-campesterol, Δ^5-stigmasterol and Δ^5-sitosterol in canola, coconut, peanut and soybean oils under simulated frying conditions at 100, 150 and 180 °C for 20 h. They found that C7 carbon is the major site of the oxygen attack. No side chain oxidation products at carbons C20 or C25 were recovered. For sterols, minor, slower oxidation including OH group at C3 carbon was also observed (Menéndez-Carreño, Ansorena, Astiasarán, Piironen, & Lampi, 2010; Smith, 1987, 1996).

The susceptibility to thermooxidation or autoxidation results from the presence of C=C double bonds, which easily undergo free radical attack followed by hydrogen abstraction on the carbon atoms in α-positions to the double bonds (Choe & Min, 2009; Gugumus, 1990, chap. 4). The hydrogen atom transfer from the molecule represents the first step of the oxidation process. Allylic hydrogens can be easily removed due to the relatively low C–H bond dissociation enthalpy (BDE) (Choe & Min, 2009). BDE represents the reaction enthalpy of homolytic abstraction of hydrogen from a molecule. The potency of an antioxidant is enhanced by relatively low bond dissociation enthalpy. BDE also correlates with the logarithm of the radical scavenging rate constants and the activation energy of the hydrogen abstraction (van Acker, Koymans, & Bast, 1993; Zhang, 2005), i.e. the lower the bond dissociation enthalpy is, the higher the reaction rate of hydrogen abstraction is.

Because phytosterols are important food components, their oxidative behaviour should be investigated. For these compounds, the thermodynamics of hydrogen atom transfer mechanism was not previously studied. Quantum chemical methods are able to provide reliable results in this field of research with minimal costs. They are useful especially in such cases, where experimental determination of the required properties or quantities is complicated. Therefore, the main aim of this work is to calculate BDEs for C–H and O–H bonds splitting-off for the relevant oxidation sites in selected Δ^5- and Δ^7-phytosterols and cholesterols (Fig. 2) using Density Functional Theory (DFT). We have studied nine Δ^5-phytosterols: Δ^5-stigmasterol, Δ^5-avenasterol, Δ^5-brassicasterol, Δ^5-epibrassicasterol, Δ^5-22-dihydrobrassicasterol, Δ^5-campesterol, Δ^5-sitosterol, Δ^5-fucosterol, Δ^5-desmosterol. We have selected also six Δ^7-phytosterols, namely: Δ^7-stigmasterol, Δ^7-avenasterol, Δ^7-campesterol, Δ^7-gramisterol, Δ^7-episterol, Δ^7-citrostadienol.

2. Computational details

All calculations were performed using Gaussian 09 program package (Frisch et al., 2009). The geometries of compounds and radical structures were optimized using DFT method with Becke's three parameter hybrid functional with Lee–Yang–Parr correlation potential (B3LYP) (Becke, 1993) without any constraints (energy cut-off of 10^{-5} kJ mol^{-1}, final RMS energy gradient under 0.01 kJ mol^{-1} Å^{-1}). The calculations were performed in a 6-31G* basis set of atomic orbitals (Rassolov, Ratner, Pople, Redfern, & Curtiss, 2001), which contains contracted Gaussian wave functions with addition of polarization functions. This basis set was selected with respect to the relatively large number of atoms in the studied molecules. The optimized structures were confirmed to be real minima by frequency analysis (no imaginary frequency). For the two sterol nuclei (Δ^5 and Δ^7) without a side chain, calculations were also performed in larger, 6-311++G**, basis set including also diffusion functions (Binkley, Pople, & Hehre, 1980), which usually provides more accurate results, however, with significantly higher computational costs.

3. Results and discussion

Bond dissociation enthalpy, BDE, is defined as

$$\mathrm{BDE} = H(\mathrm{R}^{\cdot}) + H(\mathrm{H}^{\cdot}) - H(\mathrm{R{-}H}) \quad (1)$$

where $H(\mathrm{R}^{\cdot})$ is the total enthalpy of the radical, $H(\mathrm{H}^{\cdot})$ is the total enthalpy of the abstracted hydrogen atom, and H(R–H) is the total enthalpy of the molecule. In the case of the DFT method, the total enthalpies of the species X, H(X), at temperature T are estimated from the expression

$$H(\mathrm{X}) = E_0 + \mathrm{ZPE} + \Delta H_{\mathrm{trans}} + \Delta H_{\mathrm{rot}} + \Delta H_{\mathrm{vib}} + RT \quad (2)$$

where E_0 is the calculated total electronic energy, ZPE stands for zero-point energy, $\Delta H_{\mathrm{trans}}$, ΔH_{rot}, and ΔH_{vib} are the translational, rotational and vibrational contributions to the enthalpy. Finally, RT represents the PV-work term, which is added to convert the internal energy, U, to the enthalpy ($H = U + pV$).

3.1. Bond dissociation enthalpies for Δ^5- and Δ^7-sterols nuclei

The obtained B3LYP/6-31G* O–H and C–H bond dissociation enthalpies for Δ^5-sterols and Δ^7-sterols are compiled in Tables 1 and 2, respectively. The data in Table 1 indicate that different structures of the side chain have practically no effect on the O–H, C4–H and C7–H BDEs in the Δ^5-sterols nucleus. In the case of Δ^7-sterols, the BDEs of selected bonds (O–H, C6–H, C9–H and C14–H) in their nucleus can also be considered practically identical, because they vary within 1–2 kJ mol^{-1}. The position of the C=C double bond in the two sterols nuclei does not affect the O–H BDE. This BDE is higher than all C–H BDEs in the studied molecules. In comparison to the phenolic O–H BDEs in the four tocopherols (which belong to the most effective chain-breaking antioxidants (Bauernfeind, 1980)), the O–H BDE in the studied sterols is approximately by 100 kJ mol^{-1} higher. In Klein, Lukeš, and Ilčin (2007) we found that the B3LYP/6-311++G** O–H BDEs of four tocopherols (α, β, γ, δ) lie in 297–318 kJ mol^{-1} range. The most effective α-tocopherol has the lowest O–H BDE. However, one should keep in mind that the BDE values may depend on the basis set employed for the calculation (Cabral do Couto, Costa Cabral, & Martinho Simões, 2006; Klein et al., 2007). For the sake of correctly comparing the sterols O–H BDE with the data obtained for tocopherols, the O–H and C–H BDEs for the two nuclei were also calculated in larger 6-311++G** basis set (Table 3). The comparison

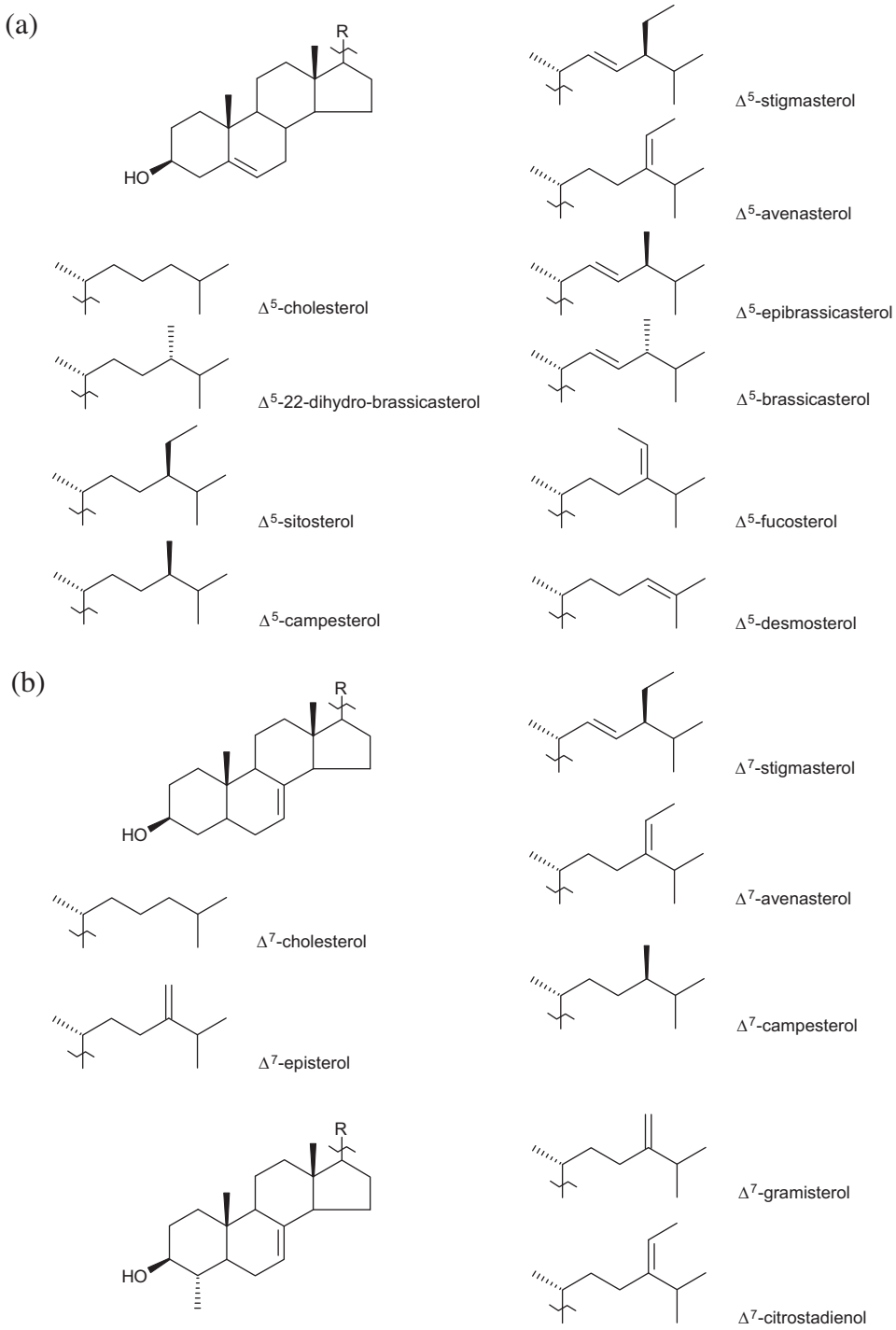

Fig. 2. Nuclei and side chains (R) of studied Δ^5-sterols (a) and Δ^7-sterols (b).

of BDEs from Tables 1–3 show that the application of larger 6-311++G** basis set leads to O–H BDEs higher by 22–24 kJ mol^{-1}.

Because no experimental or theoretical BDEs for sterols are available, we cannot assess the reliability of the obtained results directly. In the epicatechin molecule, one non-phenolic hydroxy group is present at C3 carbon (in non-aromatic C ring). B3LYP/6-311++G** dissociation enthalpy for this O–H bond reached 411 kJ mol^{-1}, while for all phenolic O–H groups in this molecule, the BDEs were in the 308–342 kJ mol^{-1} range (Vagánek, Rimarčík, Lukeš, & Klein, Unpublished results). These are in accordance with the published results of Leopoldini, Marino, Russo, and Toscano (2004). Besides, the B3LYP/6-311++G** approach provided reliable O–H BDE values for monosubstituted phenols (Klein & Lukeš, 2006) and tocopherols (Klein et al., 2007), too. Therefore, B3LYP/6-311++G** O–H BDE can be considered more reliable than the B3LYP/6-31G* ones.

On the other hand, the data in Tables 1–3 show that the two employed basis sets provide analogous C–H BDEs; found differences do not exceed 2 kJ mol^{-1}. For the C–H bond in α-position to C=C double bond in the methyloleate molecule, Pajunen, Johansson, Hase, and Hopia (2008) calculated BDE = 331 kJ mol^{-1}. This value is in good accordance with our results for hydrogen

Table 1
B3LYP/6-31G* O–H and C–H bond dissociation enthalpies (BDE) of Δ^5-sterols. The lowest value in the molecule is set in bold.

Bond	BDE (kJ mol^{-1})								
	O–H	C4–H	C7–H	C20–H	C23–H	C24–H	C24^2–H	C25–H	C26–H
Δ^5-Cholesterol	392	349	**328**	376		398		383	
Δ^5-Stigmasterol	392	349	328	**315**		317		369	
Δ^5-Avenasterol	393	349	**328**		334		341	336	
Δ^5-Brassicasterol[a]	392	349	328	**320**		321		383	
Δ^5-22-Dihydro-brassicasterol	392	349	**328**					377	
Δ^5-Campesterol	392	349	**328**			378		377	
Δ^5-Sitosterol	392	349	**329**			370		365	
Δ^5-Fucosterol	392	349	**328**		334		341	336	
Δ^5-Desmosterol[b]	392	349	328		**323**				353

[a] Identical values were found for Δ^5-epibrassicasterol.
[b] C27–H BDE = 354 kJ mol^{-1}.

Table 2
O–H and C–H bond dissociation enthalpies (BDE) of Δ^7-sterols. The lowest value in the molecule is set in bold.

Bond	BDE (kJ mol^{-1})								
	O–H	C6–H	C9–H	C14–H	C20–H	C23–H	C24–H	C24^2–H	C25–H
Δ^7-Cholesterol	393	334	325	**314**	378		398		384
Δ^7-Stigmasterol	393	334	324	**312**	316		313		369
Δ^7-Avenasterol	393	334	323	**312**		333		341	336
Δ^7-Campesterol	393	334	324	**313**					377
Δ^7-Episterol	393	334	325	**314**		332			334
Δ^7-Gramisterol	392	334	324	**313**		332			334
Δ^7-Citrostadienol	392	333	324	**312**		337		341	335

Table 3
O–H and C–H bond dissociation enthalpies (BDE) of sterols nuclei. The lowest value in the molecule is set in bold.

Bond	BDE (kJ mol^{-1})					
	O–H	C4–H	C6–H	C7–H	C9–H	C14–H
Δ^5-Sterols	415	351		**330**		
Δ^7-Sterols	416		335		328	**316**

abstraction from the $-CH_2-$ groups in α-position to a C=C double bond (C7–H in Δ^5-sterols, C23–H in the two avenasterols and Δ^5-fucosterol, C6–H in Δ^7-sterols, or C23–H in Δ^7-gramisterol, Δ^7-episterol and Δ^7-citrostadienol). In the case of 2-propene, the experimentally determined BDE for $CH_2{=}CH{-}CH_2{-}H$ bond (370 ± 2) kJ mol^{-1} was practically identical with the $C_6H_5CH_2{-}H$ BDE for toluene due to the conjugation in the subsequently formed benzyl and allyl radical (Ellison, Davico, Bierbaum, & DePuy, 1996). This experimental values are ca. 30 kJ mol^{-1} higher than the B3LYP/6-31G* BDE values for hydrogen splitting-off from methyl groups in α-position to C=C double bonds, i.e. the C24^2–H BDE in the two avenasterols, and Δ^5-fucosterol. Although the used computational approach may slightly underestimate the BDE values, it describes the observed trends correctly (Klein & Lukeš, 2006; Klein et al., 2007; Rimarčík, Lukeš, Klein, & Rottmannová, 2011). On the other hand, one should keep in mind that the obtained gas-phase values are usually close, but not necessarily identical, with the experimental solution-phase BDEs, which are, in general, higher (Klein & Lukeš, 2006; Klein et al., 2007; Rimarčík et al., 2011).

Significantly larger O–H BDEs (in comparison to C–H BDEs) are in accordance with the experimentally observed minor, slower oxidation including OH group at C3 carbon (Menéndez-Carreño et al., 2010; Smith, 1987, 1996).

The results compiled in Table 1 also confirm that in the case of intensively studied Δ^5-sterols, the C7–H bond has the lowest C–H BDE for the molecules without the C=C double bond in the side chain (Δ^5-cholesterol, Δ^5-22-dihydrobrassicasterol, Δ^5-campesterol, Δ^5-sitosterol). In Δ^5-avenasterol and Δ^5-fucosterol C7–H BDE also remains the lowest one, although in their side chains, a double bond is present. These values are in agreement with the results of experimental studies of sterols oxidation, where it was found that the C7–H bond represents the major site of oxidation attack (Johnsson, Andersson, & Dutta, 2003; Johnsson & Dutta, 2003; Lampi et al., 2002; Menéndez-Carreño et al., 2010; Oehrl et al., 2001; Smith, 1987, 1996).

Easier hydrogen abstraction from the carbon atom in the α-position to the C=C double bond due to the low C–H BDE was confirmed also for Δ^7-sterols. In this group of sterols, C14–H BDE is the lowest one (see Table 2). In all studied Δ^7-sterols, we found C14–H BDEs almost identical – they reached values in a very narrow 312–314 kJ mol^{-1} range. Unfortunately, this result cannot be validated by an experiment, since we have not found any experimental work related to Δ^7-sterols oxidation and analysis of oxidation products. When we compare the lowest B3LYP/6-311++G** C–H BDEs in Δ^5-sterol and Δ^7-sterol nuclei (Table 3), from the thermodynamics point of view, the C14–H bond in Δ^7-sterols should be even more vulnerable to oxidation attack in comparison to the C7–H bond in Δ^5-sterols. The bond dissociation enthalpies for the C4–H in Δ^5-sterols or C9–H and C6–H bonds in Δ^7-sterols, which are also in the α-position to the C=C double bonds, reached values higher by ca. 10–20 kJ mol^{-1} in comparison to the C7–H in Δ^5-sterols and C14–H bond in Δ^7-sterols.

The BDEs compiled in Tables 1–3 indicate that the Δ^7-sterol nuclei may show lower oxidation stability than the Δ^5-sterols. In other words, the Δ^7-sterols can act as more effective antioxidants from the thermodynamics point of view because of lower enthalpies of homolytic C–H bond cleavage. From the oxidation kinetics point of view, lower BDEs should correlate with higher oxidation rates.

3.2. Bond dissociation enthalpies for Δ^5- and Δ^7-sterols side chains

Experimental results (Gordon & Magos, 1983; Johnsson & Dutta, 2003; Johnsson et al., 2003; Lampi et al., 2002; Menéndez-Carreño

et al., 2010; Schroepfer, 2000; Smith, 1987, 1996) indicate that from certain carbons located in the side chains of sterols, the hydrogen atom can be easily abstracted. Since the C25–H bond in the side chains of Δ^5-sterols was often reported as an oxidation attack site (Gordon & Magos, 1983; Johnsson & Dutta, 2003; Johnsson et al., 2003; Lampi et al., 2002; Menéndez-Carreño et al., 2010; Schroepfer, 2000; Smith, 1987, 1996), the C25–H BDEs were calculated for all the studied sterols (Tables 1 and 2). These vary in relatively wide range of 334–384 kJ mol^{-1} due to the different structures of the side chains. If the C25 carbon is in the α-position to a C=C double bond, as in Δ^5-avenasterol, Δ^5-fucosterol, Δ^7-avenasterol, Δ^7-gramisterol, Δ^7-episterol and Δ^7-citrostadienol, the C25–H BDEs reach the lowest values. They are in very narrow 334–336 kJ mol^{-1} range and can be considered practically identical. On the contrary, considerably larger C25–H BDEs were found for the molecules with saturated side chains or with side chains containing C=C double bonds in other position to C25 carbon. The largest C25–H BDEs were found for the two cholesterols, Δ^5-brassicasterol and Δ^5-epibrassicasterol.

In the side chains of sterols with the lowest C25–H BDE values, also other carbon atoms are in the α-position to C=C double bond. For the majority of these C–H bonds, even lower BDEs were found. In the Δ^5-avenasterol, Δ^5-fucosterol, Δ^7-avenasterol, Δ^7-gramisterol and Δ^7-episterol, the C23–H BDEs reached values from 332 to 334 kJ mol^{-1}. Only for the Δ^7-citrostadienol the C23–H BDE is slightly higher than C25–H BDE. The lowest C23–H BDE (323 kJ mol^{-1}) was obtained for Δ^5-desmosterol, which has no hydrogen atom on the C25 carbon. In the Δ^5-desmosterol, C23–H BDE is the lowest one in the molecule. Desmosterol has two more carbons (C26 and C27) in α-positions to the C24=C25 double bond. However, in the case of these two CH_3 groups, relatively high BDE values of 353 and 354 kJ mol^{-1} were found for C26–H and C27–H bonds, respectively. In other sterols, where the CH_3 group is in α-positions to the C=C double bonds, the enthalpies of hydrogen atom abstraction are also higher than in the case of the $-CH_2-$ groups. For Δ^5-avenasterol, Δ^5-fucosterol, Δ^7-avenasterol and Δ^7-citrostadienol, which have very similar structures, the C24^2–H BDEs reached the same value, 341 kJ mol^{-1}.

In the Δ^5-brassicasterol, Δ^5-epibrassicasterol, Δ^5-stigmasterol with C22=C23 double bonds, the C20–H and C24–H bonds have the lowest dissociation enthalpies in the studied group of Δ^5-sterols. In the case of Δ^5-brassicasterol and Δ^5-epibrassicasterol C20–H and C24–H, the BDEs reached values of 320 and 321 kJ mol^{-1}, respectively. For the two stigmasterols, the BDEs for these C–H bonds vary in 313–317 kJ mol^{-1} range. Therefore, in Δ^5-brassicasterol, Δ^5-epibrassicasterol, Δ^5-stigmasterol and Δ^7-stigmasterol, the homolytic dissociation of C20–H and C24–H should be thermodynamically preferred in their side chains. However, Sims et al. (1972) found that the order of effectiveness as antioxidants in safflower oil at 180 °C was vernosterol > Δ^7-avenasterol > Δ^5-fucosterol, whilst other sterols, e.g. Δ^5-sitosterol, Δ^5-stigmasterol, Δ^7-stigmasterol or Δ^5-cholesterol, had no significant antioxidant activity. Δ^7-Citrostadienol also showed an antioxidant effect. Gordon and Magos (1983) on the basis of results presented in Sims et al. (1972) suggested the hypothesis that the antioxidant effect is greatest when free radical formation from a sterol is relatively rapid due to the presence of unhindered hydrogen atoms on an allylic carbon atom, and when the radical thus formed can isomerise to a tertiary radical, which is known to be relatively stable (Nonhebel & Walton, 1974). According Gordon and Magos (1983), the weak antioxidant effect of Δ^5-stigmasterol can be connected with a slow rate of hydrogen atoms loss at the tertiary carbon atoms because of the steric hindrance to the approach of a free radical. However, this presumption is inconsistent with the generally accepted mechanism of primary antioxidants action (Gugumus, 1990, chap. 4), where the first step of the free reactive radicals termination is the hydrogen atom transfer from the antioxidant molecule to the reactive radical intermediate – also in the case of sterically hindered phenolic antioxidants (Gugumus, 1990, chap. 4; Zhu, Zhang, & Fry, 1997).

Johnsson et al. (2003) studied the side chain autoxidation of Δ^5-stigmasterol by chromatographic and spectroscopic methods. Pure Δ^5-stigmasterol was oxidized at 120 °C for 72 h in an air-ventilated oven. Besides the common ring-structure (sterol nucleus) oxidation products, they found that 25-hydroxystigmasterol and 24-hydroxystigmasterol were also formed. Johnsson and Dutta (2003) also isolated 24-hydroxy and 25-hydroxy Δ^5-sitosterol and Δ^5-campesterol as the products of their autoxidation under the same conditions. However, for these two sterols with saturated side chains, C24–H BDEs are higher by more than 50 kJ mol^{-1} in comparison to Δ^5-stigmasterol, where the C24 carbon is in α-position to the C22=C23 double bond. The C24–H BDEs reached 370 and 378 kJ mol^{-1} in the Δ^5-sitosterol and Δ^5-campesterol, respectively. Similarly, for the tertiary carbon in the Δ^5-cholesterol side chain, we obtained C20–H BDE = 376 kJ mol^{-1}. These values are analogous to the C25–H BDEs of sterols with saturated side chains or with side chains, where the C24 carbon atom is not involved in the C=C double bond.

For the C24–H bonds in Δ^5-cholesterol, Δ^5-campesterol and Δ^5-sitosterol molecules with similar saturated side chains, we can see that BDE is the highest in the case of cholesterol (398 kJ mol^{-1}), and the lowest in Δ^5-sitosterol (370 kJ mol^{-1}), where the ethyl group is present on C24. For Δ^5-campesterol, which has the methyl group attached to C24, a 378 kJ mol^{-1} value of BDE lies between the values obtained for Δ^5-cholesterol and Δ^5-sitosterol. This comparison may indicate that in these three sterols, the C24–H bond is most labile in Δ^5-sitosterol.

Just to illustrate, how large the impact of C=C double bonds present in the sterol molecules on the C–H bond dissociation enthalpies, we have also calculated several C–H BDEs for methyl groups, e.g. C19–H and C26–H in Δ^5-cholesterol or C24^2–H in Δ^5-sitosterol, where no oxidation products were experimentally determined or expected. All these BDEs exceeded 400 kJ mol^{-1} and lie in the 406–413 kJ mol^{-1} range. In Δ^5-cholesterol, for the $-CH_2-$ groups in the side chain, the C23–H and C24–H BDEs also reached high values of 391 and 398 kJ mol^{-1}, respectively.

Although BDEs represent one of the important parameters in evaluating the antioxidant action from the thermodynamics point of view, the kinetics of the processes occurring in the system and other phenomena, including reaction environment, solubility, suitable concentration and mutual presence of other compounds, have also to be taken into account. Therefore, a reported weak antioxidant effect of stigmasterol and other sterols in safflower oil (Gordon & Magos, 1983) may result from other/consecutive reactions in the course of oxidation.

If we compare the BDE values found for the selected reaction sites in the side chains of analogous Δ^5- and Δ^7-sterols (stigmasterol, avenasterol, cholesterol and campesterol), we can see that the position of the double bond in the nucleus has a negligible effect on the C20–H and C24–H BDEs in the two stigmasterols or the C23–H and C24^2–H BDEs in avenasterols. In the case of C25–H BDE values, these can be considered independent on the C=C double bond position in Δ^5 and Δ^7 nuclei.

4. Conclusion

In this work, we have theoretically studied the O–H and C–H bond dissociation enthalpies for oxidation attack sites chosen on the basis of experimental reports on the oxidation products of sterols. For all seven Δ^7-sterols, we have found that the enthalpy of the hydrogen atom abstraction is the lowest in the case of the C14–H bond. For Δ^7-stigmasterol, almost identical BDE values

were found for the C20–H and C24–H bonds. The BDEs obtained indicate that the Δ^7-sterols may be more susceptible to oxidation attack in comparison to the Δ^5-sterols. The majority of Δ^5-sterols has the lowest BDE for the C7–H bond. However, when Δ^5-sterol has a C=C double bond in the side chain, the lowest BDE was found for the C–H bond in the α-position to the C=C bond with the exception of Δ^5-avenasterol and Δ^5-fucosterol. The homolytic dissociation of hydroxyl O–H bond requires considerably larger energy in comparison to the studied C–H bonds. Different side chains do not affect the enthalpies of the O–H and C–H bonds dissociation related to two sterols nuclei. Similarly, the position of the C=C double bond in the Δ^5 and Δ^7 nuclei does not influence the calculated BDEs related to the side chains of the C–H bonds. The obtained bond dissociation enthalpies are in accordance with published experimental data on sterol oxidation, because the C–H bonds with lowest BDE values are actually the dominant sites of oxidation attack. Sterols with lowest C–H BDEs may have the largest antioxidant potency and the ability to prevent the food from the oxidation. These show the highest ability to act as hydrogen atom donor in the process of free radicals termination.

Acknowledgements

Support by the Grant Agency of the Czech Republic via Grant No. P208/11/0161 and Swiss National Science Foundation project No. PZ00P2_132357/1 is gratefully acknowledged. This work has been also supported by the Slovak Grant Agency VEGA (Project No. 1/0137/09 and 1/0179/12) and the Science and Technology Assistance Agency (Project LPP-0230-09).

References

Bauernfeind, J. B. (1980). Tocopherols in foods. In L. J. Machlin (Ed.), *Vitamin E: A comprehensive treatise*. New York: Marcel Dekker.

Becke, A. D. (1993). Density-functional thermochemistry. III. The role of exact exchange. *Journal of Chemical Physics, 98*, 5648–5652.

Binkley, J. S., Pople, J. A., & Hehre, W. J. (1980). Self-consistent molecular orbital methods. 21. Small split-valence basis sets for first-row elements. *Journal of the American Chemical Society, 102*, 939–947.

Cabral do Couto, P., Costa Cabral, B. J., & Martinho Simões, J. A. (2006). S–H bond dissociation enthalpies: The importance of a complete basis set approach. *Chemical Physics Letters, 421*, 504–507.

Choe, E., & Min, D. B. (2009). Mechanisms of antioxidants in the oxidation of foods. *Comprehensive Reviews in Food Science and Food Safety, 8*, 345–358.

Ellison, G. B., Davico, G. E., Bierbaum, V. M., & DePuy, C. H. (1996). The thermochemistry of the benzyl and allyl radicals and ions. *International Journal of Mass Spectrometry and Ion Processes, 156*, 109–131.

Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., et al. (2009). *GAUSSIAN 09, Revision A.02*. Wallingford, CT: Gaussian, Inc.

García-Llatas, G., & Rodríguez-Estrada, M. T. (2011). Current and new insights on phytosterol oxides in plant sterol-enriched food. *Chemistry and Physics of Lipids, 164*, 607–624.

Gordon, M. H., & Magos, P. (1983). The effect of sterols on the oxidation of edible oils. *Food Chemistry, 10*, 141–147.

Gugumus, F. (1990). Stabilization of plastics against thermal oxidation. In J. Pospíšil & P. Klemchuk (Eds.). *Oxidation inhibition in organic materials* (Vol. 1). Boca Raton: CRC Press.

Johnsson, L., Andersson, R. E., & Dutta, P. C. (2003). Side-chain autoxidation of stigmasterol and analysis of a mixture of phytosterol oxidation products by chromatographic and spectroscopic methods. *Journal of the American Oil Chemists' Society, 80*, 777–783.

Johnsson, L., & Dutta, P. C. (2003). Characterization of side-chain oxidation products of sitosterol and campesterol by chromatographic and spectroscopic methods. *Journal of the American Oil Chemists' Society, 80*, 767–776.

Klein, E., & Lukeš, V. (2006). Study of gas-phase O–H bond dissociation enthalpies and ionization potentials of substituted phenols – Applicability of ab initio and DFT/B3LYP methods. *Chemical Physics, 330*, 515–525.

Klein, E., Lukeš, V., & Ilčin, M. (2007). DFT/B3LYP study of tocopherols and chromans antioxidant action energetics. *Chemical Physics, 336*, 51–57.

Lampi, A.-M., Juntunen, L., Toivo, J., & Piironen, V. (2002). Determination of thermo-oxidation products of plant sterols. *Journal of Chromatography B, 777*, 83–92.

Leopoldini, M., Marino, T., Russo, N., & Toscano, M. (2004). Antioxidant properties of phenolic compounds: H-atom versus electron transfer mechanism. *Journal of Physical Chemistry A, 108*, 4916–4922.

Menéndez-Carreño, M., Ansorena, D., Astiasarán, I., Piironen, V., & Lampi, A.-M. (2010). Determination of non-polar and mid-polar monomeric oxidation products of stigmasterol during thermo-oxidation. *Food Chemistry, 122*, 277–284.

Moreau, R. A., Whitaker, B. D., & Hicks, K. B. (2002). Phytosterols, phytostanols, and their conjugates in foods: Structural diversity, quantitative analysis, and health-promoting uses. *Progress in Lipid Research, 41*, 457–500.

Nonhebel, D. C., & Walton, J. C. (1974). *Free-radical chemistry*. Cambridge: Cambridge University Press.

Oehrl, L. L., Hansen, A. P., Rohrer, C. A., Fenner, G. P., & Boyd, L. C. (2001). Oxidation of phytosterols in a test food system. *Journal of the American Oil Chemists' Society, 78*, 1073–1078.

Pajunen, T. I., Johansson, M. P., Hase, T., & Hopia, A. (2008). Autoxidation of conjugated linoleic acid methyl ester in the presence of alpha-tocopherol: The hydroperoxide pathway. *Lipids, 43*, 599–610.

Piironen, V., Lindsay, D. G., Miettinen, T. A., Toivo, J., & Lampi, A.-M. (2000). Plant sterols: Biosynthesis, biological function and their importance to human nutrition. *Journal of the Science of Food and Agriculture, 80*, 939–966.

Rassolov, V. A., Ratner, M. A., Pople, J. A., Redfern, P. C., & Curtiss, L. A. (2001). 6-31G* basis set for third-row atoms. *Journal of Computational Chemistry, 22*, 976–984.

Rimarčík, J., Lukeš, V., Klein, E., & Rottmannová, L. (2011). On the enthalpies of homolytic and heterolytic S-H bond cleavage in para and meta substituted thiophenols. *Computational and Theoretical Chemistry, 967*(2–3), 273–283.

Schroepfer, G. J. Jr., (2000). Oxysterols: Modulators of cholesterol metabolism and other processes. *Physiological Reviews, 80*, 361–554.

Sims, R. J., Fioriti, J. A., & Kanuk, M. J. (1972). Sterol additives as polymerisation inhibitors for frying oils. *Journal of the American Oil Chemists' Society, 49*, 298–301.

Smith, L. L. (1987). Cholesterol autoxidation 1981–1986. *Chemistry and Physics of Lipids, 44*, 87–125.

Smith, L. L. (1996). Review of progress in sterol oxidations: 1987–1995. *Lipids, 31*, 453–487.

Vagánek, A., Rimarčík, J., Lukeš, V., & Klein, E. Unpublished results.

van Acker, S. A. B. E., Koymans, L. M. H., & Bast, A. (1993). Molecular pharmacology of Vitamin E: Structural aspects of antioxidant activity. *Free Radical Biology and Medicine, 15*, 311–328.

Vivacons, M., & Moreno, J. J. (2005). Beta-sitosterol modulates antioxidant enzyme response in RAW264.7 macrophages. *Free Radical Biology and Medicine, 39*, 91–97.

Wang, T., Hicks, K. B., & Moreau, R. (2002). Antioxidant activity of phytosterols, oryzanol, and other phytosterol conjugates. *Journal of American Oil Society, 79*, 1201–1206.

Yoshida, Y., & Niki, E. (2003). Antioxidant effects of phytosterol and its components. *Journal of Nutritional Science and Vitaminology, 49*, 277–280.

Zhang, H.-Y. (2005). Structure–activity relationship and rational design strategies for radical-scavenging antioxidants. *Current Computer-Aided Drug Design, 1*, 257–273.

Zhu, Q., Zhang, X. M., & Fry, A. J. (1997). Bond dissociation energies of antioxidants. *Polymer Degradation and Stability, 57*, 43–50.